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Use of Tissue Engineering Strategies to Repair Joint Tissues in Osteoarthritis: Viral Gene Transfer Approaches

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Abstract Osteoarthritis (OA) is a major chronic disease of the joints, affecting mostly the articular cartilage but also all the surrounding tissues including the subchondral bone, synovium, meniscus, tendons, and ligaments. Despite the availability in the clinic of a variety of therapeutic approaches, there is crucial need for improved treatment to protect and regenerate the cartilage with full integrity and function. In this regard, combining gene, cell, and tissue engineering-based procedures is an attractive concept for novel, effective therapy against AO, a slow, progressive, and irreversible disease. Here, we provide an overview of the treatment available for management of the progression of the OA phenotype and discuss current progress and remaining challenges for potential future treatment of patients.

Keywords Osteoarthritis · Gene therapy · Viral vectors · Tissue engineering · Cartilage repair

Introduction

Osteoarthritis (OA) is a highly prevalent, critical cause of physical disability without a definitive cure. On onset OA is mainly characterized by gradual loss of articular cartilage because of impaired anabolic and/or catabolic balance; the disease then further affects all other joint tissues (subchondral bone, synovial membrane, capsule, menisci, tendons, ligaments and periarticular muscles) [1, 2]. For patients who are too young to undergo joint replacement or for individuals at earlier stages of OA, in particular, there is a significant need to develop novel therapy to protect the cartilage, inhibiting further loss or even re-establishing its structural integrity. Such approaches as structure and/or disease-modifying drugs have not yet been successful, leaving joint arthroplasty as unmatched therapy for restoration of function and alleviation of pain. A better understanding of the factors and mechanisms leading to OA has enabled significant advances in the design of novel treatment for OA that has been tested in preclinical models. OA is a highly complex, multifactorial disease with a substantial genetic background [1, 3]. OA may be also caused by secondary issues, for example axial malalignment, loss of meniscal tissue, or repetitive stress injury. Pathological loading is another critical factor in OA [1, 4], because the response of cells in the joint to mechanical signals is impaired during OA. Obesity and production of adipokines also alter cartilage homeostasis in the joint and lead to OA [4]. OA is also strongly linked to aging processes, including mitochondrial dysfunction and changes in signaling pathways [5, 6]. Epigenetic events controlling a large group of disease-related genes have also been reported to be critically important in OA [7]. On the basis of this new knowledge, strategies using gene therapy and tissue engineering have become very attractive for developing treatments that could enable durable restoration of such joint tissues as cartilage when OA becomes irreversible (Fig. 1).

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Strategies Used to Treat OA

Target Cells

Although articular chondrocytes are the primary targets of viral gene transfer approaches to treatment of OA, other cells relevant to the pathogenesis of OA may be genetically modified to target different cellular processes or specific tissue

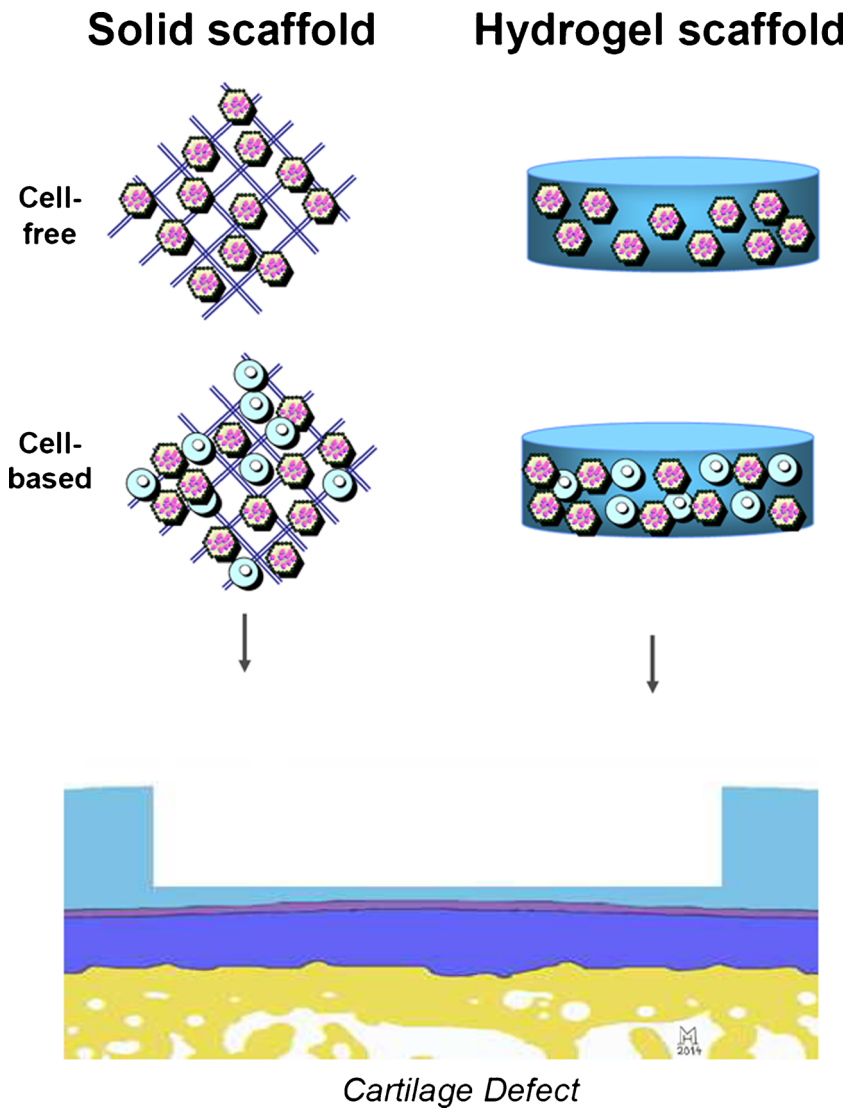

Fig. 1 Principles of gene engineering, cell engineering, and tissue engineering-based approaches for treatment of OA

types (Table 1). The many cell types present in subchondral bone are important targets for correcting the disrupted physiological relationship between the bone and the articular cartilage [2]. Pathological subchondral bone changes in OA that must be addressed include, for example, increased bone formation and turnover, changes in its microstructure, and the formation of osteophytes [2].

Target Pathways

Reproduction of the native, structural, and functional cartilage may be achieved by targeting different levels in the affected cells via:

1. inhibition of inflammatory and catabolic pathways that lead to joint surface degeneration;
2. stimulation of anabolic pathways for restoration of the damaged cartilage by promoting the production of essential components of the extracellular matrix (ECM);
3. activation of cell proliferation and survival, while preventing apoptosis and aging, to revitalize the resident cells in cartilage in the early stages of OA disease, or in the setting of transplantation;
4. prevention of endochondral ossification processes involved in osteophyte formation, vascular invasion of the cartilage at the subchondral bone junction, and cartilage calcification;
5. modulation of OA-related epigenetic events that control OA disease-related gene expression and post-translational modifications;
6. modification of the multiple genetic, or hereditary, components of OA; and
7. combination of strategies that by themselves do not enable complete reproduction of the original chondrocyte phenotype and cartilage structure.

Table 1 Target cells relevant to the pathogenesis of OA for potential genetic modification

Cells	Objective of the modification
Articular chondrocytes	Repopulate the affected cartilage surface; increase ECM biosynthesis
Osteocytes	Reconstruct the subchondral bone
Osteoclasts	Inhibit OA bone resorption
Periosteal cells	Inhibit osteophyte formation at joint margins
MSCs	Enhance potential (cartilage and/or bone) for chondrogenesis and/or osteogenesis
iPSCs	Enhance potential (cartilage and/or bone) for chondrogenesis and/or osteogenesis
ESCs	Enhance potential (cartilage and/or bone) for chondrogenesis and/or osteogenesis
Meniscal cells	Inhibit OA effects on meniscal cells
Ligament cells	Inhibit OA effects on ligament cells
Tenocytes	Inhibit OA effects on tenocytes
Muscle cells	Inhibit OA effects on muscle cells

MSCs, mesenchymal stem cells; iPSCs, inducible pluripotent stem cells; ESCs, embryonic stem cells.

Choice of a Gene Transfer Vector

A variety of viral vectors are available to achieve this objective, each of which has advantages and limitations because of the biology of the viruses from which they are derived (Table 2).

Adenoviral Vectors

Adenoviral vectors enable very high transduction efficiencies and levels of transgene expression in vitro (more than 80 % and close to 100 %) [8–41], but serious concerns remain about their safety because of immunogenicity in vivo and short-term efficacy (1 to 2 weeks maximum), which are critical issues in the context of a slow, progressive disease such as OA.

Retroviral Vectors

These vectors can become integrated into the host genome, enabling long-term maintenance of the transgenes delivered. However, integration may lead to insertional mutagenesis and activation of tumorigenic genes. Furthermore, retroviral vectors can only transduce dividing cells and at relatively low efficacy (<20 % before cell selection) [12–14, 24, 28, 42–44, 45•, 46–52], making them unsuitable for targeting adult chondrocytes with low proliferative activity.

Lentiviral Vectors

Lentiviral vectors are good alternatives, because they can become integrated into the genome of nondividing cells, and have higher levels of transduction (at least 70 %) [53•, 54, 55•, 56], although concerns remain about their potential for insertional mutagenesis.

Recombinant Adeno-Associated Virus (rAAV) Vectors

rAAV are derived from a nonpathogenic, replication-defective human parvovirus and are much less immunogenic than adenoviruses. Stable, episomal rAAV transgenes are expressed with high efficiency (>65 %) in dividing and nondividing cells for extended periods of time (at least 150 days in situ) and can access the cells via dense ECM [33, 57–87]. The use of self-complementary AAV (scAAV) has enabled, at least in part, circumvention of the step-limiting conversion of single-stranded into double-stranded DNA [64, 66, 67, 69, 70, 86]. Trans-splicing systems have also been used successfully to improve the size capacity of the vectors [88]. For these various reasons, rAAV has become the vector of choice for clinical applications.

Use of Biocompatible Materials

The advantage of using a biomaterial to treat joint disorders is that it enables spatially and temporarily controlled delivery and expression of the candidate therapeutic gene to the sites of injury. Interestingly, despite the availability of many biocompatible materials in research on this topic, relatively few have been used for treatment of OA as opposed to focal articular

Table 2 Overview of currently used viral gene transfer vectors for OA

Class	Advantages	Limitations	Integration
Adenovirus	• Very high efficiency	• Potential replication competence • Toxicity • Immunogenicity • Short-term transgene expression	No
Retrovirus or lentivirus	• High efficiency • Long-term transgene expression	• Potential replication competence • Risk of insertional mutagenesis	Yes
Recombinant adeno-associated virus	• Very high efficiency • Long-term transgene expression • Low immunogenicity	• Difficult to produce • Size limitation • Potential serotype-restricted cell specificity	Mostly episomal

cartilage defects. They mostly include collagen gels [89, 90] and hyaluronic acid [91].

Evidence in Vitro

Whereas most of the vectors mentioned above have been used successfully to transduce most, if not all, of the cells relevant to the pathogenesis of OA in experimental systems in vitro, thus far, only rAAV vectors are capable of modifying the cells in situ when they are located in their natural ECM environment. Table 3 gives an overview of the combined strategies currently used in vitro.

Inhibition of Inflammatory and Catabolic Pathways

Many studies focusing on limiting or blocking cartilage loss have been performed in vitro or in vivo, using viral vectors to drive expression of inhibitors of matrix-degrading enzymes and of inflammatory pathways (IL-1Ra, sIL-1R, IL-1-specific shRNA, sTNFR, TIMPs, IκBα, NF-κBp65-specific siRNA, kallistatin, thrombospondin-1, pro-opiomelanocortin, Dkk-1, ADAMTS-5-specific siRNA, heme oxygenase-1) [8, 11, 17, 19, 20, 35, 49, 52, 54, 55•, 66, 69, 71, 77, 79, 80, 86]. Their effects may be enhanced by using a three-dimensionally woven, porous, biomimetic poly(ε-caprolactone) (PCL) scaffold [55•]. Alternatively, such chondroprotective cytokines as IL-4 and IL-10 can be delivered in viral vectors [52].

Stimulation of Anabolic Pathways

Successful activation of anabolic processes has been reported upon viral delivery of enzymes that produce or process ECM components [15], growth factors, including IGF-I, FGF-2, BMPs, TGF-β, GDF-5, HGF, PTHrP, Indian hedgehog (IHH), scleraxis [16–18, 22, 25, 26, 29–32, 36–38, 40, 41, 43, 47, 48, 53•, 58, 60, 64, 78, 83, 87, 92]. Because many of these are involved in endochondral ossification during skeletal development, osteophyte formation, cartilage calcification, and abnormal bone changes may occur in vivo. Scaffolds such as PCL scaffold [53•], or tissue-specific transcription factors (SOX5, SOX6, SOX9) [9, 21, 23, 24, 45•, 51, 59, 63, 85] have been used to enhance anabolism.

Activation of Cell Proliferation and Survival—Prevention of Apoptosis and Aging

The restoration or activation of cell vitality and proliferation can be achieved by application of IGF-I, FGF-2, BMPs, TGF-β [58, 60–62, 72, 87, 92], telomerase (hTERT) [44, 50, 56], or inhibitors of apoptosis (kallistatin) [20].

Prevention of Osteophyte Formation and Cartilage Vascular Invasion

Studies in vivo have provided antagonists of the TGF-β/BMP pathway, for example latency-associated peptide and inhibitory Smads, which inhibit osteophyte formation [34]. Inhibition of vascular invasion has been attempted using sFlt-1, a soluble receptor that acts a vascular endothelial growth factor antagonist, preventing angiogenesis and cartilage resorption and resulting in persistent cartilage regeneration and repair in a rat model of OA [48]. Similar results were obtained when applying other types of inhibitor of angiogenesis, for example thrombospondin-1, leading to reduced microvessel density, inflammation, and suppression of the progression of OA in a model of anterior cruciate ligament transection (ACLT) in rats [19]. Gene transfer of chondromodulin has also been reported to inhibit the invasion of vessel structures, endochondral ossification, and terminal chondrocyte hypertrophy in porcine cartilage lesions while stimulating chondrogenic differentiation and the formation of hyaline-like matrix in the lesions [70]. Also, remarkably, application of pro-opiomelanocortin (POMC), a precursor of neuropeptides with potent anti-inflammatory activity, has been shown to suppress microvessel density, reduce NF-κB activity, and prevent the progression and severity of ACLT-induced OA in rats [35].

Modulation of OA-Related Epigenetic Events

With the identification of functional miRNAs, new molecular therapy can be envisaged, for example delivery of miRNAs to modulate the production of proinflammatory cytokines [93],

Table 3 Overview of current OA treatments using viral gene transfer and tissue engineering approaches

Model	Method	Biomaterial	System	Ref.
In-vitro	Lentiviral IL-1Ra transduction of bone marrow-derived MSCs	PCL scaffold	Resistance to inflammation challenge (IL-1)	[55•]
	Lentiviral TGF-β transduction of bone marrow-derived MSCs	PCL scaffold	Cartilage ECM formation	[53•]
In-vivo	Retroviral SOX trio co-transduction of adipose-derived stem cells	Fibrin glue	Healing and prevention of degenerative changes in surgically induced OA (rats)	[45•]

down-regulate the expression of matrix-degrading enzymes [94], or up-regulate type-II collagen expression [95]. Also, remarkably, gene transfer of sirtuin 1, a histone deacetylase, has been shown to protect chondrocytes under stress conditions [96, 97]; it thus has strong promise as a new therapeutic approach.

Modification of Genetic Factors in OA

Therapy compensating for loss of function or inhibiting undesirable gene overexpression in OA might be envisaged but, so far, little work has been performed to address this crucial issue.

Combined Approaches

Successful co-transfer of different factors has been achieved by providing combinations of activators of anabolic and proliferative processes [21, 61], inhibitors of catabolic pathways [52], or inhibitors of catabolism with activators of anabolic and proliferative pathways [17, 27, 30]. Another interesting approach has been developed recently on the basis of co-application of anabolic factors with specific silencers of potentially undesirable cellular processes, for example a combination of TGF-β with a small hairpin RNA to silence type-I collagen expression and thus minimize the formation of fibrocartilage [98].

Evidence in Vivo

Gene engineering and tissue engineering-based treatment of OA in vivo might, in theory, be achieved either by providing a biomaterial coated with a gene-transfer vector (direct approach) or by using a scaffold carrying or seeded with genetically modified cells (indirect approach). Direct procedures are simpler and more convenient, because they are less invasive, yet indirect strategies might be desirable in cases of advanced, severe OA in which little cartilage surface and few chondrocytes remain and when cell repopulation is required.

In contrast with the literature on focal cartilage defects [9, 45•, 99–103], few studies have examined the benefits of applying viral gene transfer methods concomitantly with a biocompatible material (hydrogel compounds or solid scaffolds) to treat experimental models reflective of the complex pathology of OA. So far, only Lee et al. [45•] have demonstrated the benefits of injecting adipose-derived stem cells that had been retrovirally-co-transduced with the SOX trio and suspended in fibrin glue to prevent the progression of degenerative changes in surgically induced OA in rats (Table 3). Therapeutic approaches for OA in vivo have, instead, focused on administration of gene transfer vectors [11, 19, 20, 34, 35, 66, 80, 104] and of genetically modified cells [48, 52, 105] in the absence of supportive matrices.

Conclusions

Because of the remarkable advances in experimental research in cell biology, molecular biology (therapeutic candidate factors and genes), biomaterials, and translational science, gene engineering and tissue engineering-based strategies are attractive approaches to repair of joint tissues in OA. There is a large body of evidence showing the benefits of gene therapy for OA, including two clinical trials of indirect administration of retrovirally-modified, TGF-β-expressing chondrocytes [106] and direct delivery of IL-1Ra via rAAV [107]. However, little is known about the value of combining such a methods with use of biocompatible materials both in vitro and in experimental models of the disease in vivo compared with current knowledge on the advantages of this approach for focal cartilage defects. Among unanswered questions, the choice of an appropriate scaffold for treatment of large OA lesions compared with defined focal defects may be the most complex to address, because such issues as the best source of cells, candidate gene, and vector (most likely rAAV) have been, in general, well investigated. It will also be important to keep in mind that the products used to generate a new treatment will need challenging approval by the regulatory organizations before use in a clinical procedure. Only a combined effort among scientists, clinicians, industry, and regulatory organizations will enable us to address the crucial issue of treating the slow, progressive disease in OA patients.

Compliance with Ethics Guidelines

Conflict of Interest Magali Cucchiarini and Henning Madry declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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